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HBRC Journal

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Application of microbial biocementation to improve the physico-mechanical properties of cement mortar

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Received 1 March 2012; accepted 30 April 2012

KEYWORDS

Biocement;
Cement mortar;
Bacterial cells;
Calcium carbonate

Abstract Calcite is one of the most common and wide spread mineral on Earth constituting 4 wt% of the Earth's crust. It is naturally found in extensive sedimentary rock masses, as lime stone marble and calcareous sandstone in marine, fresh water and terrestrial environments. Calcium carbonate is one of the most well known mineral that bacteria deposit by the phenomenon called biocementation or microbiologically induced calcite precipitation (MICP). Such deposits have recently emerged as promising binders for protecting and consolidating various building materials. Microbially enhanced calcite precipitation on concrete or mortar has become an important area of research regarding construction materials. This study describes a method of strength and water absorption improvement of cement–sand mortar by the microbiologically induced calcium carbonate precipitation. A moderately alkalophilic aerobic *Sporosarcina pasteurii* was incorporated at different cell concentrations with the mixing water. The study showed that a 33% increase in 28 days compressive strength of cement mortar was achieved with the addition of about one optical density (1 OD) of bacterial cells with mixing water. The strength and water absorption improvement are due to the growth of calcite crystals within the pores of the cement–sand matrix as indicated from the microstructure obtained from scanning electron microscopy (SEM) examination.

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Introduction

Portland cement concrete has clearly emerged as the material of choice for the construction in the world today. This is mainly due to low cost of materials and construction for concrete structure as well as low cost of maintenance.

Microorganisms and microbially mediated mineralization processes are active in almost every environment on earth [1,2] and possibly in extraterrestrial systems as well [3]. In natural environments, chemical CaCO_3 precipitation ($\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3$) is accompanied by biological

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Peer review under responsibility of Housing and Building National Research Center.



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processes, both of which often occur simultaneously or sequentially. Microbes from soils and aqueous media have been frequently reported to induce the precipitation of calcium carbonate mineral phases in both natural and laboratory settings [4].

Microbial mineral precipitation technologies have already been used for sand consolidation [5] and improvement strength of bricks [6].

Recently, an inherent cement based biomaterial has been developed to remediate the cracks and fissures in concrete structures [7–8]. Previous studies have been shown that the addition of specific microorganisms to cement–sand mortar or concrete deposit inorganic substances inside the pores of the matrices, which can be used as a filling material to remediate cracks within the structures [9,10]. It was also noted that the addition of bacterial cells with growth media, composes of 25 Mm calcium chloride and 20 g/l urea, to the mortar/concrete could increase the compressive strength (20–35%) of the material with respect to control mix [10–12]. On the other hand, no significant increase in strength produced when growth media without bacterial cells was added to cement mortar [13]; due to little amount of calcium chloride in growth media.

The biologically induced cement based material thus also exhibited better durability and crack repairing performance compared to normal concrete materials [14].

This investigation aims to study the effect of microbial induced calcite precipitation to improve the physico-mechanical properties of cement mortar.

Experimental

Materials

Microorganisms

Bacteria species which called *Sporosarcina pasteurii* (previously known as *B. pasteurii*) NCIMB 8841, obtained from National Collection of Industrial and Marine Bacteria-England by national research center, was used in this study. The aerobic organisms is a moderately alkalophilic (growth optimum pH 9.25) and it was shown that sufficient activity for biocementation could be cultivated in non sterile conditions with a minimum of upstream and downstream processing. *S. pasteurii*, a common bacterium naturally occurring in the subsurface, is such an aerobic micro-organism. In addition, cells of *S. pasteurii* do not aggregate; this ensures a high cell surface to volume ratio, a condition that is essential for efficient cementation initiation.

Culture media

S. pasteurii was cultivated under aerobic batch conditions in 10 g/l yeast extract, 5 g/l NaCl, 25 mM CaCl₂ and 20 g/l urea. Medium pH adjusted before sterilization to 6.5 by 1 N HCl. Urea/CaCl₂ was added post autoclaving by 0.22 filter sterilization to prevent chemical decomposition under autoclave condition [15].

Cement and sand

The starting materials used in this investigation are ordinary Portland cement (OPC) which provided by Suez Cement Company, El-Suis governorate, Egypt and sand obtained from El-Wasta Area, Beni-Suif governorate, Egypt. Their chemical compositions are given in Table 1 and the mineral composition of sand is shown in Fig. 1. The sand composes mainly of quartz(Q) in addition to small amount of feldspar(F).

Methods of investigation

Preparation of microbial cement mortar

The mortar was prepared using sand: cement ratio 3:1 by weight. On inch cubic molds were prepared. Sand and cement were thoroughly mixed, adding along with grown culture, at a W/C ratio of 0.46, of *S. pasteurii* correspondence to OD at 600 nm of 0.5, 1.0 and 1.5 OD; bacterial cells concentration was measured by spectrophotometer at wave length 600 nm. The fresh mortar pastes were cast into the mold and compacted on a vibration machine then cured in humidity chamber with relative humidity 100% for 24 h. After de-molding the control specimens were cured under tap water and the specimens with bacteria were cured under solution of 20 g/l urea and 25 mM CaCl₂ at room temperature until the times of testing at the intervals of 3, 7, 14 and 28 days.

Water absorption

Water absorption measurements were done by weighing the saturated specimens (W_1) and dried specimens in oven at 80 °C for 24 h (W_2) at curing times of 3, 7, 14 and 28 days.

The water absorption is calculated from the following equation:

$$\text{Water absorption, \%} = [(W_2 - W_1)/W_1] \times 100.$$

Compressive strength

This test was carried out on four specimens following the procedure described by ASTM [16]. Compressive strength measurements were carried out using five tones German Brűf Pressing Machine with a loading rate of 100 kg/min.

Scanning electron microscopy (SEM)

The scanning electron micrographs of freshly fractured specimens were taken with *Inspect S* (FEI Company, Holland) equipped with an energy dispersive X-ray analyzer (EDAX) at the accelerating voltage of 200 V to 30 kV.

Results and discussion

Water absorption

Water absorption of cement mortar (CM) specimens cured under tap water and CM mixed with bacterial cell concentrations of optical densities (0.5, 1 and 1.5 OD) cured under growth

Table 1 Chemical composition of starting materials, wt.%.

Oxide (%)	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	Na ₂ O	K ₂ O	SO ₃	TiO ₂	P ₂ O ₅	L.O.I	Total
Cement	21.09	4.79	3.65	61.78	2.71	0.22	0.12	3.11	–	–	2.50	99.97
Sand	97.78	0.88	0.45	0.03	0.01	0.25	0.10	0.01	0.10	0.03	0.23	99.89

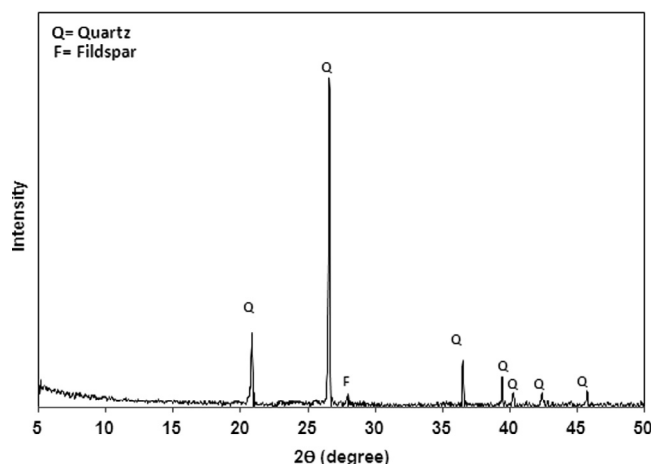


Fig. 1 XRD diffractogram of sand.

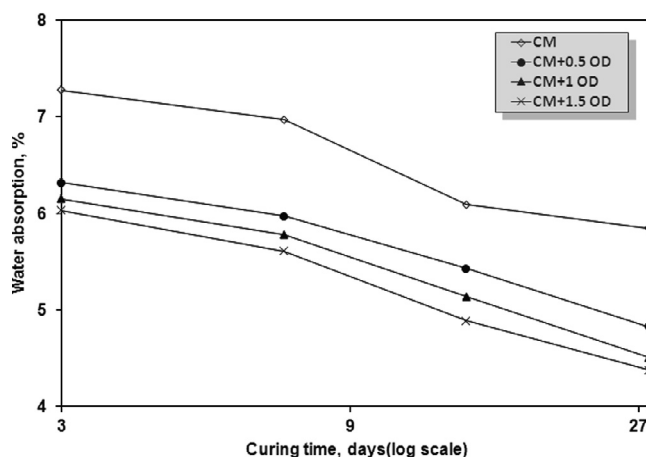


Fig. 2 Water absorption of cement mortar with and without bacterial cells up to 28 days.

media, at 3, 7, 14 and 28 days, are graphically represented in Fig. 2. The results show that the water absorption of cement mortar with or without bacterial cells decreases with time of curing up to 28 days; this is due to the continuous hydration and accumulation of hydrated products which fill the open pores of the specimens. Also, the water absorption values of CM specimens mixed with bacterial cells at all OD concentrations (0.5, 1, and 1.5) are lower than those of control specimens. This is attributed to that bacterial biomass and microbial calcite precipitation within the pores and on the surface of CM. On the other hand, the water absorption values of cement mortar decrease with OD of bacterial cells. Evidently, with increasing bacterial cells concentrations, the biomass and precipitated calcite content increases which fill some of the open pores and therefore, decreases the extent of water absorption. The water absorption is linearly proportional to the total porosity of the cement mortar.

Compressive strength

The compressive strength of CM specimens cured under tap water and CM specimens mixed with bacterial cells and cured

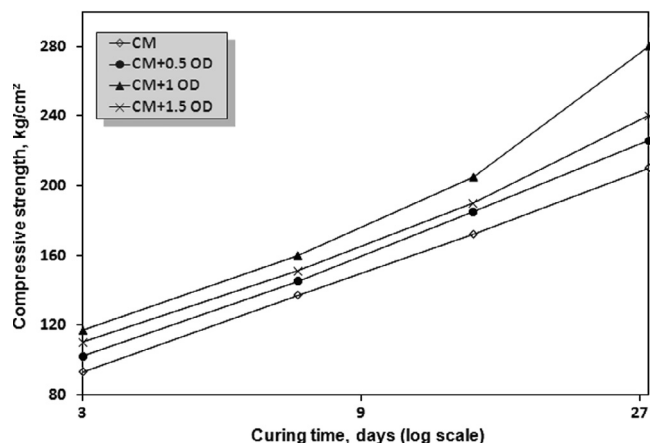


Fig. 3 Compressive strength of cement mortar with and without bacterial cells up to 28 days.

under growth media are graphically represented as a function of curing time in Fig. 3. Obviously, the strength values of CM specimens mixed with bacterial cells are higher than those of control CM. In addition, the overall trend of an increase in compressive strength up to 28 days might be attributed to the behavior of microbial cells within the cement mortar matrix. During the initial curing period, microbial cells obtained good nourishment, because the cement mortar was still porous; but its growth might not be proper due to the completely new environment for microbes. It may also be possible that as the pH of the cement remained high, cells were at inactive conditions and as the curing period was increased, it started growing slowly. Upon cell growth, calcite would have precipitated on the cell surface as well as within the cement mortar matrix. Once many of the pores in the matrix were plugged, the flow of the nutrients and oxygen to the bacterial cells stopped, eventually the cells either died or turned into endospores and acted as an organic fibers; this is associated with the increase of compressive strength of the mortar cubes. This explains the behavior of the relatively higher compressive strength value after 28 days in case of cement mortar cubes prepared with microbial cells. There was a measurable increase in compressive strength of cement mortar cubes prepared with *S. pasteurii* in this study was in agreement with the result reported in previous studies [17,18]. Thus, it was concluded that the increase in compressive strength is mainly due to consolidation of the CM matrix with a consequent filling of the pores inside the CM cubes with microbiologically induced calcium carbonate precipitation. On the other side, the compressive strength of CM increases with concentration of bacterial cells up to 1 OD. This is mainly due to the microbial precipitation of calcite crystals and the consequent filling of open pores. The compressive strength of CM specimens mixed with 1 OD, which give about 33% improvement of compressive strength than control sample at 28 days of curing, is greater than that of CM specimens mixed with 1.5 OD. This is due to that the precipitation rate of calcium carbonate increases with the concentration of bacterial cells up to 1.5 OD with the increase of the amorphous fraction of calcium carbonate, deposited within the pores of CM matrix, leading to a decrease the extent of the improvement of compressive strength.

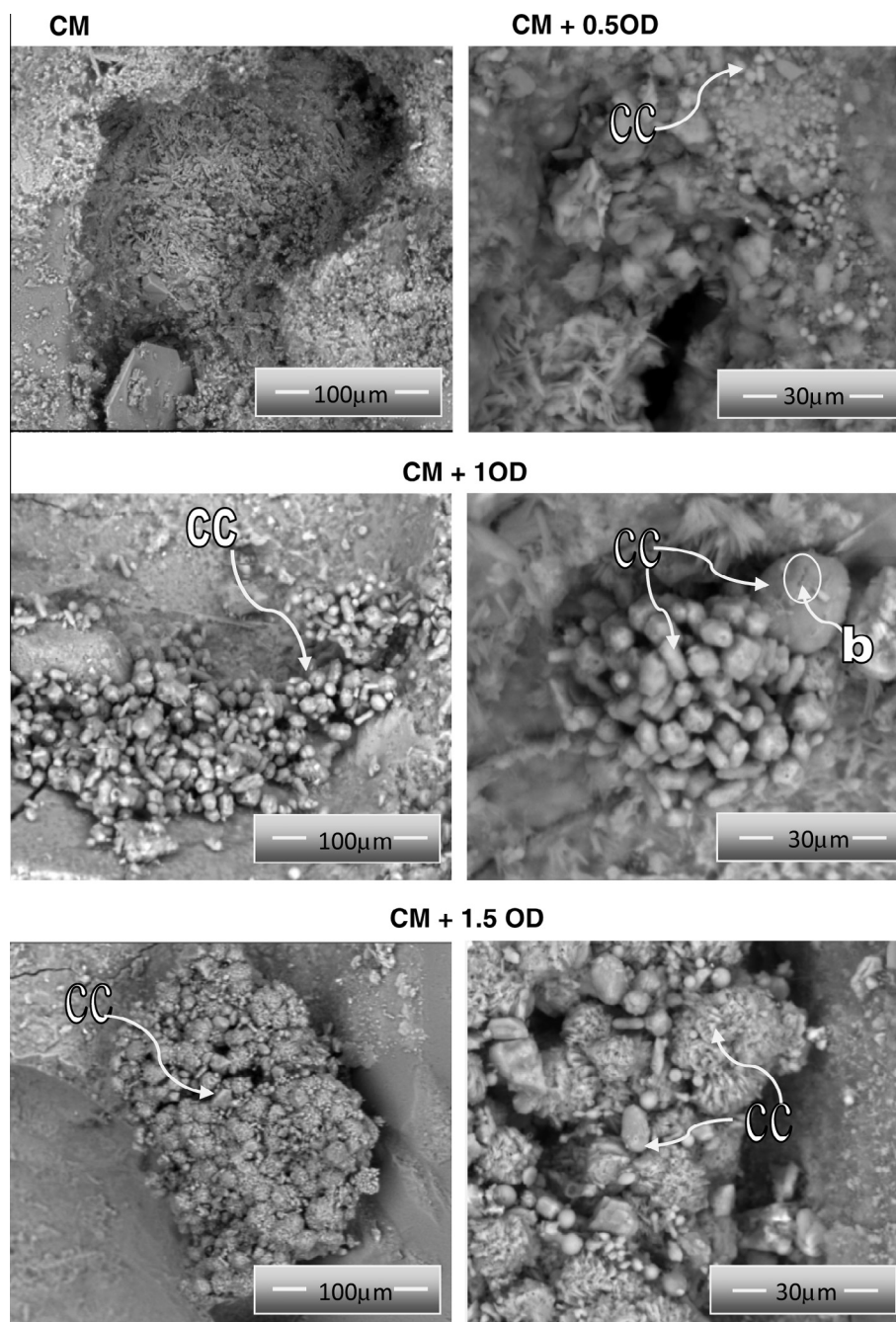


Fig. 4 SEM of control CM and CM mixed with 0.5 OD, 1 OD and 1.5 OD of bacterial cells after 28 days.

These results will receive further confirmation from those of microstructure obtained from SEM micrographs reported later in this investigation (Fig. 4).

Scanning electron microscopy (SEM)

The SEM micrograph of control CM or CM mixed with 0.5, 1 and 1.5 OD bacterial cells up to 28 days are shown in Fig. 4. From SEM observations, calcium carbonate (CC) precipitated by bacterial cells could be clearly distinguished within the pores of CM matrix; meanwhile, the CC phase isn't observed in control CM specimens made without any addition of bacterial cells. On the other hand, the precipitated calcium carbon-

ate content increases with bacterial cells concentration. Evidently, SEM micrographs exhibited different morphologies and sizes of calcium carbonate crystals in CM specimens made with different concentrations of bacterial cells; spherical calcite crystals precipitated by 0.5 OD of bacterial cells which possess smaller size than that of rod shape and spherical calcite crystals precipitated by 1 OD of bacterial cells. In addition, the amorphous calcite phase and little amount of spherical calcite crystals are observed in CM mixed with 1.5 OD. The bacterial cell with rod shape (b) is observed as a hole distinguished inside the surface of spherical calcite crystal. EDAX analysis indicated that the new phase precipitated, by bacterial cells, in CM matrix is calcium carbonate (Fig. 5).

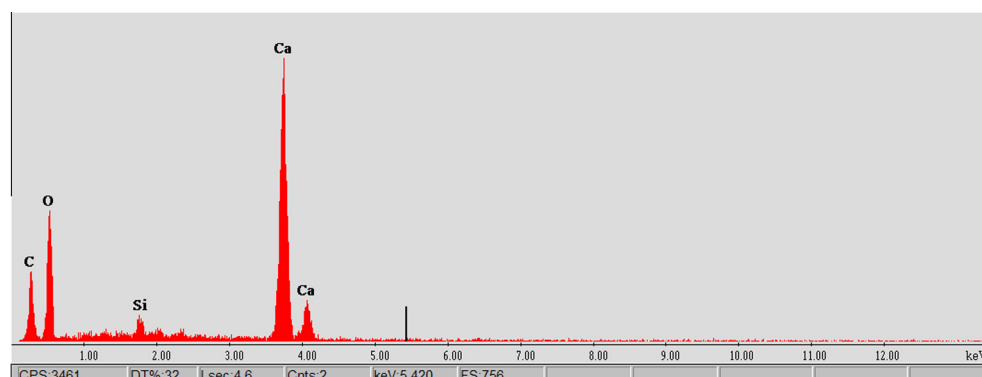


Fig. 5 Energy dispersive X-ray analysis (EDAX) of precipitated calcite crystals in CM by bacterial cells.

Conclusion

Based on the result of the present study, it is concluded that the physico- mechanical properties, compressive strength and water absorption, of the bacteria modified mortar were improved due to the deposition of the new calcite material by the bacterial activity. The water absorption of cement mortar decreases with concentration of bacterial cells while the compressive strength increases with concentration of bacterial cells up to 1 OD; while a decrease in strength improvement was observed when cement mortar mixed with 1.5 OD of bacterial cell. Therefore, the optimum bacterial cells concentration which leads to the highest improvement in mortar gives higher compressive strength and lower water absorption is 1 OD. The degree of crystallinity of calcite crystals precipitated by 1 OD in cement mortar is higher than that precipitated by 1.5 OD of bacterial cells. Also, the amount and size of calcite crystals precipitated in cement mortar by 1 OD is greater than that precipitated by 0.5 OD of bacterial cells.

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